

19. Federal  
Republik

(Fed. Eagle)

of Germany  
Patent Office

12. Document of Disclosure  
11. DE 39 26 287 A1

21. File No.: P 39 26 287.1  
22. Day of Application: 08/09/89  
43. Day of Disclosure: 02/21/91

61. Int. Cl. (7):  
A61K37/02  
A61 K 35/60

Stamp of British Library  
13 MAR 1991

71. Applicant:  
Clasbrummel, Bernhard, 7800 Freiburg, FRG

72. Inventor:  
same as applicant

74. Patent Agent:  
Müller, B., Dipl.-Chem. Dr. rer. nat., patent attorney,  
8031 Seefeld

54. Medical Application of omega-Conotoxin GVIA or omega-Conotoxin-GVIA Analogs for  
Sympatholysis

Medical use of omega-Conotoxin GVIA or a omega-Conotoxin GVIA analog for sympatholysis in a limited area of the body. Fields of application are ailments of an extremity or of an organ, or part of an organ, respectively, where a sympathetic dysregulation or a reduced perfusion or a hypoxia are assumed in their etiopathogenesis.

BEST AVAILABLE COPY

- Column 1 -

## Specification

The invention concerns the medical application of omega-Conotoxin GVIA (omega-CT) or omega-Conotoxin GVIA analogs (omega-CT-A) for sympatholysis according to the above mentioned requirements.

Up to now omega-CT is used as a "tool" in research. The basic idea for the invention is the application of omega-CT or of an omega-CT-A in a limited area of the body; thus a local sympatholytic effect is achieved but a systemic toxic effect is avoided.

At present the peripheral intravenous sympatholysis with guanethidin is carried out for therapy of sympathetic reflex dystrophy (Blumberg, H.; 1988. Development and therapy of the pain syndrome in case of sympathetic reflex dystrophy. *Pain*, 2, 125-143) and in primary fibromyalgia (Bengtsson, A. and Bengtsson, M.; 1988. Regional sympathetic blockade in primary fibromyalgia. *Pain*, 33, 161-167). Guanethidin inhibits sympathetic stimulation (main effect), the transport of noradrenaline into the presynaptic vesicles (Uptake-block and storage discharge) and after chronic administration develops a supersensitivity particularly for noradrenaline.

Introduction of the new substance: Omega-CT, a basic, arginin-rich peptide toxin of the marine snail *Conus geographus*, blocks the transmitter release in various parts of the nervous system. Responsible for this inhibitory process is probably a blockade of voltage-sensitive calcium channels (VSCCs), which can be distinguished at the sensitive dorsal root ganglion (DRG) into T-, L- and N-types (Miller, R. J.; 1987. Multiple calcium channels and neuronal function, *Science*, 235, 46-52). Omega-CT shows an inhibition of L- and N-VSCCs at the DRG. Organic  $\text{Ca}^{2+}$ -agonists and antagonists like dihydropyridins influence only L-VSCCs.

Voltage measurements at the neuromuscular endplate of the frog muscle proved an omega-CT mediated reduction of acetylcholine release (Sano et al.; 1987. Effects of synthetic omega-Conotoxin, a new type  $\text{Ca}^{2+}$  antagonist, on frog and mouse neuromuscular transmission, *Eur. J. Pharmacol.* 141, 235-241). No effect of omega-CT could be demonstrated at the mouse diaphragm.

Omega-CT centrally blocks the electrically evoked release of tritium-marked acetylcholine and noradrenaline in the rabbit's hippocampus.  $^{45}\text{Ca}^{2+}$  flux in cerebral synaptosomal preparations of the rat are reduced after omega-CT administration as well as the calcium intake in cultured neurons of the chick's cerebrum.

A study in cooperation with the inventor (Clasbrummel et al., 1989. Inhibition of noradrenaline release by omega-Conotoxin GVIA in the rat tail artery. *Br. J. Pharmacol.*, 96, 101-110) showed that omega-CT inhibits the electrically evoked vasoconstriction and noradrenaline release, respectively, in the system of the sympathetically innervated rat tail artery. At the same organ Verapamil does not influence the noradrenaline ...

... release (Zsoter et al., 1984. Effects of Verapamil on [ $^3\text{H}$ ]norepinephrine release. J. Cardiovasc. Pharmacol., 6, 1060-1066).

**Assumed effects/interactions of omega-CT are:**

- presynaptic, sympathetic N-calcium channel blockade and following release inhibition of noradrenaline,
- total blockade after 10 min.; after 1 hr. absence of toxin minimal recovery; own preliminary experiments showed 50-75% recovery after 20 hrs.,
- total inhibitory effect is achieved with 1,5-log-steps (advantage: a 30-fold dilution of a 10 nanomolar solution remains without effect),
- can be antagonized by calcium,
- effect can be antagonized by noradrenaline and vasopressine.

According to this, omega-CT is probably more specific and more effective than guanethidine for the application in the peripheral intravenous sympatholysis and has a better therapeutical breadth due to the full inhibitory effect within 1.5 log-steps (Clasbrummel et al., 1989. Inhibition of noradrenaline release by omega-Conotoxin GVIA in the rat tail artery. Br. J. Pharmacol., 96, 101-110).

**Further differences:**

- duration of effect    guanethidine: 2-3 weeks;  
                                  omega-CT: ca. 1 day
- guanethidine is an established substance; omega-CT is possibly  
allergenic.

The systemic application of protamine (basic, arginin-rich peptide from the sexual organ of the salmon trout), of a variety of peptide-chemotherapeutics as well as the local administration of botulinus toxin for the Meige-syndrome, spasmus facialis (Poeck, K., 1987, Neurology, Springer-Verlag, Berlin-Heidelberg-New York-London-Paris-Tokyo, 7. Auflage, 326, 380) and torticollis spasticus may make the use of omega-CT seem possible from an immunological and toxicological point of view.

Botulinus toxin is a highly potent peptide toxin (MG 140 000 - 150 000) of the bacteria Clostridium botulinum and inhibits the ACh-release at the motor endplate.

Example for the peripheric intravenous sympatholysis (PIVS):

Analogous to the peripheral sympatholysis with guanethidine or analogous to the peripheral intravenous local anesthesia (Larsen, R., 1987. Anesthesia. Urban and Schwarzenberg, Munich-Vienna-Baltimore, 2. edition, 355-357) 0,05 mg (0.01 to 0.4 mg) omega-CT or a omega-CT-A, diluted in NaCl-solution 0.9%, shall be administered.

Optimal is the combination with Na-EDTA (10 to 400 mg). Thus the calcium concentration in the extremity shall be temporarily lowered to 1 to 2 mmol/l, resulting in better peptide binding.

Another combination with a local anesthetic (Lidocaine 2%, 3 to 15 ml) for the temporary pain therapy and for the compensation of a membrane destabilisation due to reduced calcium concentration is quite conceivable.

Other fields and techniques of application ...

### **- Column 3 -**

... for omega-CT or an omega-CT-A emerge from the above mentioned requirements.

### **Patent claims**

1. Medical application of omega-Conotoxin GVIA (omega-CT) or an omega-Conotoxin-analog (omega-CT-A) for sympatholysis.
2. Use according to claim 1., characterized by the sympatholysis being a peripheric intravenous sympatholysis (PIVS).
3. Use according to claim 1., characterized by the sympatholysis being a peripheric intraarterial sympatholysis (PIAS).

4. Use according to one of the claims 1. to 3., characterized by omega-CT or an omega-CT-A being administered in combination with the sodium salt of ethylene diamine tetraacetic acid (Na-EDTA).
5. Use according to one of the claims 1. to 3., characterized by omega-CT or an omega-CT-A being administered in combination with a local anesthetic.
6. Use according to one of the claims 1. to 3., characterized by omega-CT or an omega-CT-A being administered in combination with EDTA and a local anesthetic.
7. Use according to one of the claims 5. and 6., characterized by the local anesthetic being Lidocaine.
8. Use according to one of the claims 1. to 3., characterized by the sympatholysis being employed for therapy of the sympathetic reflex dystrophy (SRD).
9. Use according to one of the claims 1. to 3., characterized by the sympatholysis being employed for therapy of the primary and secondary Raynaud-syndrome.
10. Use according to one of the claims 1. to 3., characterized by the sympatholysis being employed for therapy of primary fibromyalgia.
11. Use according to one of the claims 1. to 3., characterized by the sympatholysis being employed for therapy of arterial occlusion in stages III/IV as described by FONTAINE.
12. Use according to one of the claims 1. to 3., characterized by the sympatholysis being employed for increase of blood circulation after hand-, foot-surgery or after skin transplantation.
13. Use according to one of the claims 1. to 3., characterized by the sympatholysis being employed for diagnostic sympatholysis.
14. Use according to claim 1., characterized by omega-CT or an omega-CT-A being injected intraarterially or being administered by controlled infusion, respectively, upstream of a vessel segment which is to be treated.
15. Use according to one of the claims 1. and 14., characterized by the vessel segment which is to be treated being located distal of the branching of the Arteria renalis.
16. Use according to one of the claims 1. ...

- Column 4 -

... and 14., characterized by the vessel segment which is to be treated being located distal of the branching of an Arteria coronaria.

17. Use according to one of the claims 1. and 14., characterized by the vessel segment which is to be treated being located distal of the branching of an Arteria mesenterica.
18. Use according to one of the claims 1. and 14., characterized by the vessel segment which is to be treated being located distal of the branching of a cerebral artery.
19. Use according to one of the claims 1. and 14., characterized by the vessel segment which is to be treated being located distal of the branching of the Arteria brachialis or femoralis.
20. Use according to one of the claims 1. and 14., characterized by omega-CT or an omega-CT-A being administered in combination with a thrombolyticum.

Annotation by translator:

In claims 15. -20. the translation "Use according to one of the claims 1. *and* 14., ..." is exact. It appears likely, however, that the author intended to say "Use according to one of the claims 1. *to* 14., ...".

⑫ BUNDESREPUBLIK  
DEUTSCHLAND



DEUTSCHES  
PATENTAMT

⑫ **Offenlegungsschrift**  
⑪ **DE 39 26 287 A 1**

⑤ Int. Cl. 5.  
**A61 K 37/0**  
A 61 K 35/60

⑳ Aktenzeichen: P 39 26 287.1  
㉑ Anmeldetag: 9. 8. 89  
㉒ Offenlegungstag: 21. 2. 91

㉑ Anmelder:  
Clausbrummel, Bernhard, 7800 Freiburg, DE

㉒ Vertreter:  
Müller, B., Dipl.-Chem. Dr.rer.nat., Pat.-Anw., 8031  
Seefeld

㉓ Erfinder:  
gleich Anmelder

㉔ Medizinische Anwendung von omega-Conotoxin GVIA oder omega-Conotoxin GVIA-Analoga zur Sympathikolyse

Medizinische Verwendung von omega-Conotoxin GVIA oder einem omega-Conotoxin GVIA-Analogen zur Sympathikolyse in einem begrenzten Körperbereich. Anwendungsgebiete sind Krankheiten einer Extremität oder eines Körperorgans bzw. Körperorganteils, in deren Ätiopathogenese eine sympathische Dysregulation oder eine Minderperfusion oder eine Hypoxie angenommen wird.

DE 3926287 A1

Die Erfindung betrifft die medizinische Anwendung von omega-Conotoxin GVIA (omega-CT) oder omega-Conotoxin GVIA-Analoga (omega-CT-A) zur Sympathikolyse nach den obengenannten Ansprüchen.

Bisher wird omega-CT als "Werkzeug" in der Forschung benutzt. Grundidee der Erfindung ist die Anwendung von omega-CT oder eines omega-CT-A in einem begrenzten Körperbereich; so wird eine lokal-sympathikolytische Wirkung erreicht, eine systemisch-toxische Wirkung jedoch vermieden.

Die periphere intravenöse Sympathikolyse wird derzeit mit Guanethidin zur Therapie der sympathischen Reflexdystrophie (Blumberg, H.; 1988. Zur Entstehung und Therapie des Schmerzsyndroms bei der sympathischen Reflexdystrophie. Der Schmerz, 2, 125—143) und bei der Primären Fibromyalgie (Bengtsson, A. and Bengtsson, M.; 1988. Regional sympathetic blockade in primary fibromyalgia. Pain, 33, 161—167) durchgeführt. Guanethidin hemmt die sympathische Stimulation (Hauptwirkung), den Transport von Noradrenalin in die präsynaptischen Vesikel (Uptake-Block sowie Speicherentleerung) und entwickelt nach chronischer Gabe besonders für Noradrenalin eine Supersensitivität.

Vorstellung der neuen Substanz: Omega-CT, ein basisches, argininreiches Peptidgift der Meeresschnecke *Conus Geographus*, hemmt die Transmitterfreisetzung in verschiedenen Bereichen des neuronalen Systems. Verantwortlich für diesen Hemmprozeß ist wahrscheinlich eine Blockierung von spannungsabhängigen Kalziumkanälen (voltage sensitive calcium channels, VSCCs), die am sensiblen Wurzelganglion (dorsal root ganglion, DRG) in T-, L- und N-Typen unterschieden werden können (Miller, R. J.; 1987. Multiple calcium channels and neuronal function. Science, 235, 46—52). Omega-CT zeigt am DRG eine Hemmung von L- und N-VSCCs. Organische  $Ca^{2+}$ -Agonisten und Antagonisten wie z. B. Dihydropyridine beeinflussen nur L-VSCCs.

Potentialmessungen an der neuromuskulären Endplatte des Frostmuskels wiesen eine omega-CT vermittelte Verminderung der Acetylcholinfreisetzung nach (Sano et al.; 1987. Effects of synthetic omega-conotoxin, a new type  $Ca^{2+}$  antagonist, on frog and mouse neuromuscular transmission. Eur. J. Pharmacol., 141, 235—241). Am Mausdiaphragma konnte keine Wirkung von omega-CT gezeigt werden.

Zentral hemmt omega-CT im Hippocampus des Kaninchens die elektrisch evozierte Freisetzung von tritiummarkiertem Acetylcholin und Noradrenalin.  $^{45}Ca^{2+}$  Fluxes in cerebralen Synaptosomenpräparaten der Ratte sind nach omega-CT Gabe reduziert sowie auch die Kalziumaufnahme in kultivierte Neurone des Hühner-cerebrums.

In postganglionär sympathischen Nerven sind L-VSCCs nur gering vertreten, da Dihydropyridine die Neurotransmitterfreisetzung nicht oder nur in hohen Konzentrationen ( $\approx 100 \mu\text{mol/l}$ ) beeinflussen (Godfraind et al., 1986. Calcium channel antagonism and calcium entry blockade. Pharmacol. Rev., 38, 321—416).

Eine Untersuchung unter Mitarbeit des Erfinders (Clasbrummel et al.; 1989. Inhibition of noradrenaline release by omega-conotoxin GVIA in the rat tail artery. Br. J. Pharmacol., 96, 101—110) zeigte, daß omega-CT am System der sympathisch innervierten Rattenschwanzarterie die elektrisch evozierte Vasokonstriktion bzw. Noradrenalinfreisetzung hemmt. Verapamil beeinflusst am selben Organ die Noradrenalinfreiset-

zung nicht (Zsoter et al., 1984. Effects of verapamil on  $[^3H]$ noradrenaline release. J. Cardiovasc. Pharmacol., 6, 1060—1066).

Anzunehmende Wirkungen/Wechselwirkungen von omega-CT sind:

- präsynaptische, sympathische N-Kalziumkanalblockade und folgende Freisetzungshemmung von Noradrenalin,
- voller Hemmeffekt nach 10 min; nach einer Stunde Toxinabwesenheit minimale Erholung; eigene orientierende Versuche zeigten nach 20 Stunden 50—75% Erholung,
- volle Hemmwirkung wird über 1,5 Log-Stufen erreicht (Vorteil: eine 30fache Verdünnung einer 10 nanomolaren Lösung bleibt ohne Effekt)
- Antagonisierbarkeit durch Kalzium
- Effekt ist durch Noradrenalin und Vasopressin antagonisierbar

Omega-CT ist hiernach für die Anwendung in der peripheren intravenösen Sympathikolyse wahrscheinlich spezifischer und wirkungsvoller als Guanethidin und hat eine bessere therapeutische Breite aufgrund der vollen Hemmwirkung nach 1,5 Log-Stufen Clasbrummel et al.; 1989. Inhibition of noradrenaline release by omega-conotoxin GVIA in the rat tail artery. Br. J. Pharmacol., 96, 101—110).

Weitere Unterschiede:

- Wirkungsdauer Guanethidin: 2—3 Wochen; omega-CT: ca. 1 Tag
- Guanethidin ist eine etablierte Substanz; omega-CT ist möglicherweise allergen.

Die systemische Anwendung von Protamin (basisches, argininreiches Peptid aus dem Geschlechtsapparat des Seelachses), verschiedenster Peptid-Chemotherapeutika sowie die lokale Gabe von Botulinustoxin beim Meige-Syndrom, Spasmus facialis (Poeck, K.; 1987. Neurologie. Springer-Verlag, Berlin-Heidelberg-New York-London-Paris-Tokyo, 7. Auflage, 326, 380) und Torticollis spasticus läßt eine Verwendung von omega-CT aus immunologischer und toxikologischer Sicht für möglich erscheinen.

Botulinustoxin ist ein hochpotentes Peptidgift (MG 140 000—150 000) des Bakteriums *Clostridium botulinum* und hemmt die ACh-Freisetzung an der motorischen Endplatte.

Beispiel für die periphere intravenöse Sympathikolyse (PIVS):

Analog der peripheren Sympathikolyse mit Guanethidin oder analog der peripheren intravenösen Lokalanästhesie (Larsen, R.; 1987. Anästhesie. Urban und Schwarzenberg, München-Wien-Baltimore, 2. Auflage, 355—357) sollen 0,05 mg (0,01 bis 0,4 mg) omega-CT oder ein omega-CT-A, gelöst in NaCl-Lsg. 0,9%, gegeben werden.

Optimal ist die Kombination mit Na-EDTA (10 bis 400 mg). Die Kalziumkonzentration soll so temporär in der Extremität auf 1 bis 2 mmol/l erniedrigt werden, woraus eine verbesserte Peptidbindung resultiert.

Eine weitere Kombination mit einem Lokalanästhetikum (Lidocain 2%, 3 bis 15 ml) zur temporären Schmerztherapie und zum Ausgleich einer Membranstabilisierung aufgrund verminderter Kalziumkonzentration ist gut denkbar.

Andere Anwendungsbereiche und Anwendungstech-



**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☐ BLACK BORDERS

☒ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES

☐ FADED TEXT OR DRAWING

☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING

☐ SKEWED/SLANTED IMAGES

☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS

☐ GRAY SCALE DOCUMENTS

☐ LINES OR MARKS ON ORIGINAL DOCUMENT

☒ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY

☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**